

1 **Food-chain transfer of zinc from contaminated *Urtica dioica* and *Acer***  
2 ***pseudoplatanus* L. to the aphids *Microlophium carnosum* and**  
3 ***Drepanosiphum platanoidis* Schrank**

4 Danielle Sinnett<sup>ab\*</sup>, Tony R. Hutchings<sup>a</sup> and Mark E. Hodson<sup>b</sup>

5 <sup>a</sup>Centre for Forestry and Climate Change, Forest Research, Alice Holt Lodge, Farnham,  
6 Surrey, GU10 4LH, UK.

7 <sup>b</sup>Department of Soil Science, School of Human and Environmental Sciences, University of  
8 Reading, Whiteknights, Reading, RG6 6DW, UK.

9 \*Corresponding author. Forest Research, Alice Holt Lodge, Farnham, Surrey, GU10 4LH,  
10 UK. Tel: +44 1420 22255 Fax: +44 1420 520180

11 Email addresses: [danielle.sinnett@forestry.gsi.gov.uk](mailto:danielle.sinnett@forestry.gsi.gov.uk), [tony.hutchings@forestry.gsi.gov.uk](mailto:tony.hutchings@forestry.gsi.gov.uk),  
12 [m.e.hodson@reading.ac.uk](mailto:m.e.hodson@reading.ac.uk)

**Abstract**

This study examines the food-chain transfer of Zn from two plant species, *Urtica dioica* (stinging nettle) and *Acer pseudoplatanus* (sycamore maple), into their corresponding aphid species, *Microlophium carnosum* and *Drepanosiphum platanoidis*. The plants were grown in a hydroponic system using solutions with increasing concentrations of Zn from 0.02 to 41.9 mg Zn/l. Above-ground tissue concentrations in *U. dioica* and *M. carnosum* increased with increasing Zn exposure ( $p < 0.001$ ). Zn concentrations in *A. pseudoplatanus* also increased with solution concentration from the control to the 9.8 mg Zn/l solution, above which concentrations remained constant. Zn concentrations in both *D. platanoidis* and the phloem tissue of *A. pseudoplatanus* were not affected by the Zn concentration in the watering solution. It appears that *A. pseudoplatanus* was able to limit Zn transport in the phloem, resulting in constant Zn exposure to the aphids. Zn concentrations in *D. platanoidis* were around three times those in *M. carnosum*.

**Capsule**

Concentrations of Zn in two aphid species are dependant on species and exposure.

**Keywords**

Stinging nettle; Sycamore maple; Common nettle aphid; Sycamore aphid; Contaminated land

## 1. Introduction

The importance of the impact of contaminated land on terrestrial ecological receptors is increasingly being recognised in site investigation, risk assessment and remediation processes. Practitioners commonly use an Ecological Risk Assessment (ERA) to determine the potential for harm that a site may pose to ecological receptors and many countries have produced frameworks and guidance for conducting such investigations (Bryns and Crane, 2002). The ERA process often makes use of a combination of field and laboratory analysis and models to determine the risk to either ecological function or the food-chain transfer of pollutants. The majority of the ecotoxicological tests used in ERA are based on ecological function and use ecological endpoints such as mortality, reproduction and growth. In order to estimate the risk to higher organisms from a contaminated site it is often necessary to use models to predict the pollutant concentrations through the food-chain and relate these to published toxicological endpoints for the species of interest. There are a variety of models available to estimate the food-chain transfer of pollutants (USEPA, 2002; USEPA, 2003). However, the models are often not species specific, may have been based on aquatic organisms (that may not be appropriate for flying insects), or may only be applicable to a certain group of contaminants (USEPA, 2002; USEPA, 2003). This has serious implications for those using such models to estimate risk from contaminated land to ecological receptors. At best it may result in significant gaps in the range of biological species for which such a risk assessment can be conducted. At worst it may result in an over- or under-estimation of the risk leading to either unnecessarily costly remediation or no remediation taking place where it is needed.

*Urtica dioica* L. is prevalent in almost all urban ecosystems and is an early coloniser of contaminated land (Barta and Cagán, 2003; Edwards et al., 1998). It is extremely important in urban ecosystems as it provides a habitat and food source for a wide range of invertebrates (Barta and Cagán, 2003; Davis, 1991). In addition, it is also relatively simple to cultivate, widely available and fast growing (Davis, 1991), and as such, may be a useful species for ecotoxicological testing (Sinnott et al., In press). *Acer pseudoplatanus* L. is a tree species that has been introduced to the UK, but is commonly found in urban areas (Gilbert, 1991). It is an early coloniser (Dixon, 2005) and tolerant of a wide range of site conditions

60 (Moffat and McNeill, 1994). *U. dioica* and *A. pseudoplatanus* both have extremely prevalent  
 61 species-specific aphids associated with them; *Microlophium carnosum* Buckton and  
 62 *Drepanosiphum platanoidis* Schrank respectively.

63 The food-chain transfer of metals to a variety of aphids have been assessed in a  
 64 number of studies (Crawford et al., 1995; Green et al., 2003; Merrington et al., 1997a),  
 65 although these studies have all concentrated on aphids whose hosts are agricultural plant  
 66 species. Aphids are an important source of food for a large number of other insects and  
 67 birds, either indirectly for their honeydew (e.g. ants) or directly (e.g. parasitoids, ladybirds and  
 68 blue tits; Braun and Flückiger, 1985; Gilbert, 1991). *M. carnosum* is a large aphid (3.3 to 3.8  
 69 mm) commonly found on *U. dioica*, primarily on the underside of the leaves and the stem  
 70 (Rotheray, 1989) during May to October (Barta and Cagán, 2003; Davis, 1991). *D.*  
 71 *platanoidis* is abundant on the underside of leaves of *A. pseudoplatanus*, during April to  
 72 October, with population peaks in June and October (Dixon, 2005). The importance of aphids  
 73 as a food source may mean that they could provide a pathway for metal contamination to  
 74 transfer to higher organisms. Elevated populations of some aphid species (*Aphis pomi*) have  
 75 also been reported close to roads, and it has been suggested that this may be, in part, due to  
 76 the increased concentrations of Zn in the host plant as this is an essential element for aphids  
 77 (Kean and Müller, 2004). Used in conjunction with *U. dioica* and *A. pseudoplatanus*, *M.*  
 78 *carnosum* and *D. platanoidis* have the potential to assess the risk of food-chain transfer of  
 79 metals in urban ecosystems.

80 This study aims to assess the transfer of Zn to the aphids *M. carnosum* and *D.*  
 81 *platanoidis* from *U. dioica* and *A. pseudoplatanus* grown under hydroponic conditions in order  
 82 to determine the potential for Zn transfer to aphids in urban ecosystems. The study was  
 83 originally carried out with Cd in addition to Zn, but the small masses of aphids combined with  
 84 the smaller concentrations of Cd in their tissue meant that Cd concentrations in aphids were  
 85 often below detection limits and therefore Cd data are not reported here due to the patchy  
 86 nature of the dataset.

## 2. Materials and methods

### 2.1. Transfer of *Zn* into *Microlophium carnosum* and *Drepanosiphum platanoidis*

*U. dioica* were cultivated in the same way as described in Sinnott et al. (In press). *U. dioica* cuttings, taken from Alice Holt Forest, Farnham, UK, and *A. pseudoplatanus* (bare rooted 1+1 stock; Prees Heath Forest Nurseries, Shropshire, UK) were planted individually in 1 litre containers filled with perlite. Perlite was used as it has no inherent sorption capacity that could influence Zn availability. Additionally, pores between individual perlite beads ensure an aerobic environment. Pea shingle was placed on the perlite to a depth of 2 cm to minimise evaporation.

A fully replicated randomised block experiment with five plants per Zn treatment was set up in a glasshouse. Plants were grown under 16 h of artificial light and 8 h darkness per day (photosynthetically active radiation = 0.37 mmol/m/s). The temperature of the glasshouse was regulated to 20 °C ( $\pm 5$  °C) by heating and ventilation.

Each container was watered with one of five solutions: control ( $\frac{1}{4}$  strength Hoagland's solution for *A. pseudoplatanus* and full strength for *U. dioica* (Hoagland and Arnon, 1941) or one of four Zn treatments in Hoagland's solution. The Hoagland's formulation provided background micronutrient concentrations of 0.02 or 0.08 mg Zn/l for the  $\frac{1}{4}$  strength or full strength solutions respectively. Zn amendments were added as  $\text{ZnSO}_4 \cdot 7\text{H}_2\text{O}$  to provide concentrations of 0.02 (control), 5, 10, 20, and 50 mg Zn/l. The solution in each container was replaced by mass when necessary to return to a given mass.

*M. carnosum* were added to the *U. dioica* pots 26 d after planting whilst *D. platanoidis* were added to the *A. pseudoplatanus* pots 84 d after planting. Differences in timing were due to the availability of sufficient aphid populations in the field. Leaves with aphids on them were removed from Alice Holt Forest and placed at the base of each plant. Enough leaves were used so that at least 5 aphids were transferred to each pot. Each plant was then covered individually with a fine mesh net suspended from the ceiling. This was tied securely around the lip at the top of the pot to prevent the aphids from moving to different plants. At each watering, the netting was loosened round the pot and lifted enough to add the appropriate solution and re-secured.

*U. dioica* and *A. pseudoplatanus* were harvested 28 and 14 d respectively, after the aphids had been added. *D. platanoidis* populations appeared to be declining on the *A. pseudoplatanus* so these were harvested earlier than *U. dioica* in order to ensure that enough aphid mass was available for analysis. Reproduction rates of *D. platanoidis* vary during the season, being closely linked to the amino-nitrogen content of the leaves and this decline is likely to have been a result of the leaves reaching maturity (Dixon, 2005). The netting was loosened from around the pot and the stem cut, the netting was then closed at the bottom and detached from the ceiling, the netting along with its contents were then placed in the freezer at -20 °C for 2 h. The plants were then removed from the freezer and the dead aphids collected individually with a fine brush and tweezers. *A. pseudoplatanus* were divided into their stem, petiole and leaf components. After thawing, the above-ground tissue of *U. dioica* and leaf and shoot tissues of *A. pseudoplatanus* were washed in deionised water to remove the honeydew, weighed and dried at 70 °C for 24 h and reweighed. The stem tissues of *A. pseudoplatanus* were discarded as the aphids only feed on the petioles and leaves. The aphids were weighed, dried at 50 °C for 24 h and re-weighed. The aphid and plant material samples were then milled and analysed to determine their Zn concentrations.

## 2.2. Determination of phloem or water extractable plant Zn concentrations

In order to understand the Zn exposures to the aphids, a further experiment was set up to determine the phloem Zn concentrations in *A. pseudoplatanus* and the water extractable plant Zn concentrations in *U. dioica*. *U. dioica* cuttings and *A. pseudoplatanus* were planted individually in 1 litre containers filled with perlite in the same way as for the aphid exposure experiment. A fully replicated randomised block experiment with five replicates for both *U. dioica* and five replicates for *A. pseudoplatanus* was set up in a glasshouse under the same conditions as the aphid exposure experiment. Each container was watered with one of three solutions: control (¼ strength Hoagland's solution for *A. pseudoplatanus* and full strength for *U. dioica*) or one of two Zn treatments in Hoagland's solution of 0.02 (control), 5, and 20 mg Zn/l.

*U. dioica* and *A. pseudoplatanus* were harvested after the same duration as the aphid experiment in order to ensure that the plants had been exposed to the Zn solutions for the

same time period. The method used to determine the concentration of Zn in the sycamore phloem tissue was based on that of Thornber and Northcote (1961) which extracts the water-soluble material within the phloem. The leaf and shoot tissues of *A. pseudoplatanus* were removed from the stem tissue and discarded. The bark was carefully removed from the stem tissue using a grafting knife and the phloem tissue was then removed, again with a grafting knife. The phloem tissue was weighed and then boiled at 100 °C in 200 ml of deionised water for 3 h. Following boiling, the samples were centrifuged and the solution removed and filtered through a 0.45 µm Whatman filter. It was not possible to separate the phloem tissue of *U. dioica* from the rest of the stem so the entire above-ground biomass was subjected to boiling in order to derive the water-soluble fraction of the plant material. The above-ground tissue of *U. dioica* was removed, weighed and boiled at 100 °C in 300 ml of deionised water for 3 h. The solutions were then analysed to determine their Zn concentrations.

### 2.3. Determination of Zn concentration

The Zn solutions used for watering and the phloem extracts were analysed, with yttrium as an internal standard, using a Spectro Flame Inductively Coupled Plasma – Optical Emission Spectrometer (ICP-OES; Spectro Analytical Instruments, West Midlands, UK). The target Zn concentrations in the solutions used for watering of 0.02, 5, 10, 20, and 50 mg Zn/l were found to be 0.02, 4.7, 9.8, 18.0 and 41.9 mg Zn/l respectively.

Bush branches and leaves (NCS DC73349, China National Analysis Centre for Iron and Steel) and oriental tobacco leaves (CTA-OTL-1, Commission for Trace Analysis of the Committee for Analytical Chemistry of the Polish Academy of Sciences and Institute of Nuclear Chemistry and Technology, Warsaw, Poland) were used as certified reference materials (CRM) during the analysis of the plant tissue. Plant samples and the CRMs were prepared for analysis by dry-ashing at 450 °C for 18 h and wet digestion (Chapman, 1967). Wet digestion was achieved by incubating each sample for 1 h at 60 °C in 0.75 ml concentrated HNO<sub>3</sub>, followed by a further 14 h incubation with 2.25 ml concentrated HCl and heating for 2 h at 110 °C. After cooling, 0.15 ml of 30% H<sub>2</sub>O<sub>2</sub> was added to each sample followed by heating for 0.5 h at 110 °C. To ensure complete oxidation of all organic matter the H<sub>2</sub>O<sub>2</sub> treatment was performed twice. The digested samples were analysed for Zn using

the ICP-OES (Kilbride et al., 2006); the limit of detection was 0.67 µg/kg for Zn. Mean recovery was 104.9 and 98.6% from the bush branches and leaves and oriental tobacco respectively.

Mussel (CE278, European Commission, Geel, Belgium) and bovine liver (1577b, US Department of Commerce, National Institute of Standards and Technology, Gaithersburg, MD 20899, USA) tissues were used as CRMs during analysis of the aphid samples. Aphid samples and CRMs were digested in 1 ml concentrated HNO<sub>3</sub> at 180 °C for 1 h, after which 1 ml of deionised water was added and the sample further digested at 180 °C to dryness. A further 0.01 ml of concentrated nitric acid was added and the sample digested at 60 °C for 1 h. The digested samples were analysed for Zn using the ICP-OES (Kilbride et al., 2006). Mean recovery from mussel and bovine liver was 92.9 and 93.6% respectively for the *M. carnosum* samples and 111.8 and 102.6% respectively for the *D. platanoidis* samples.

#### 2.4. Statistical analysis

The plant and aphid Zn uptake data were subjected to general linear regression analysis to assess the significance of changes in plant and aphid concentrations with increasing Zn concentration in hydroponic solutions and plant material respectively, using Genstat version 8.1 (Genstat, 2005). Mean values are reported with ± standard errors throughout.

Linear and exponential models of Zn uptake into each of the plant and aphid tissue types compared to that of the solution concentration and, in the case of aphids, the leaf concentrations were fitted using Genstat version 8.1 (Genstat, 2005). A comparison of the residual sum of squares of alternative models relative to the smallest residual mean square was used to determine the most appropriate model. This comparison was used for nested models and was referred to an F-distribution with 1, *n* degrees of freedom where *n* is the residual degrees of freedom from the exponential model.

### 3. Results

Zn concentration in solution had a significant effect on the Zn uptake into the above ground tissue ( $F_{1,13}=533.63$ ;  $p<0.001$ ) of *U. dioica*; no plants survived in the 41.9 mg Zn/l solution treatment (Fig. 1). Zn concentration in both solution and nettle tissue had a



significant effect on the Zn concentration in *M. carnosum* ( $F_{1,13}=107.95$ ;  $p<0.001$  and  $F_{1,13}=77.38$ ;  $p<0.001$  respectively; Fig. 1). The concentration of water-soluble Zn in *U. dioica* increased significantly with increasing Zn concentration in solution ( $F_{1,13}=138.89$ ;  $p<0.001$ ) from 6.6 to 47.6 mg/kg (Fig. 2). The difference between the bulk tissue concentration of Zn and that which was water-soluble increased with increasing Zn exposure; the water-soluble fraction being 2 to 17 times smaller than the bulk tissue concentration.

Zn concentration in solution had a significant effect on the Zn uptake into the leaf ( $F_{2,22}=3.57$ ;  $p=0.046$ ) and shoot ( $F_{2,22}=5.43$ ;  $p=0.012$ ) tissue of *A. pseudoplatanus*; this relationship was exponential not linear (Fig. 3). There was no significant effect of the concentration of Zn in solution or in the petiole or leaf tissue of *A. pseudoplatanus* on the concentration in *D. platanoidis* using either the linear or exponential models (Fig. 3). Similarly, the concentration of Zn in the phloem extract was not significantly related to the concentration of Zn in solution (Fig. 2). Phloem concentrations in *A. pseudoplatanus* were smaller than those in the leaf and shoot tissues. This difference increased with increasing Zn concentrations; phloem concentrations being 11 to 25 times lower than leaf or shoot tissue concentrations.

The concentration of Zn in the above-ground tissue of *U. dioica* were approximately 13 times that in the leaves of *A. pseudoplatanus* as a result of exposure to the 18.0 mg Zn/l solution;  $2153\pm68.7$ mg/kg compared with  $163\pm20.6$  mg/kg. Despite this, the Zn concentration in *M. carnosum* was less than a third of that in *D. platanoidis*;  $131.5\pm11.0$  mg/kg compared with  $406\pm21.2$  mg/kg.

#### 4. Discussion

No *U. dioica* survived in the 41.9 mg/l solution, in contrast Sinnett et al. (In press) found that *U. dioica* were able to survive, albeit with reduced growth, in this solution. This difference is likely to be as a result of the additional stress exerted on the plants by the introduction of the aphids. Zn concentrations in the above-ground tissue of *U. dioica* increased with Zn exposure, reaching a mean of approximately 2100 mg/kg for the 18.0 mg Zn/l solution. This is in the range that was reported in a separate experiment where *U. dioica* were exposed to Zn under the same experimental conditions, but in the absence of aphids;

where concentrations of Zn in the leaf, shoot and stem tissue were approximately 1800, 2800 and 2500 mg/kg respectively (Sinnott et al., In press). In *A. pseudoplatanus*, tissue concentration increased up to the 9.8 mg Zn/l solution and then remained constant at approximately 160 mg/kg despite the increasing Zn concentration in solution. Zn concentrations in the above-ground tissue of *U. dioica* have been reported to range between 42 and 52 mg/kg in uncontaminated soils (Hobbelen et al., 2004). Leaf concentrations of between 23 and 532 (mean 113 mg/kg) have been reported in *U. dioica* growing on dredged sediments with a Zn concentration of between 149 and 1817 (mean 54 mg/kg; Tack and Verloo, 1996). Zn concentrations in *U. dioica* around the Avonmouth smelter have been found to be as high as 3000 mg/kg, although this is likely to have occurred from atmospheric deposition as well as soil uptake (Jones, 1991). The substantial quantities of Zn that nettles appear to be capable of taking up make this species an important pathway for Zn in the food-chain. Mertens et al. (2004) found Zn concentrations with a mean of 74 mg/kg in *A. pseudoplatanus* grown on dredged sediments with a Zn concentration of 359 mg/kg. The normal range of Zn in plant tissue has been reported to be 27-150 mg/kg with an upper toxic limit of 100-500 mg/kg (Kabata-Pendias and Pendias, 2001), which suggests that the concentrations reported here for *A. pseudoplatanus* are unlikely to cause a toxic effect.

The Zn concentrations in the tissue of *U. dioica* and *A. pseudoplatanus* showed large differences; at the lowest Zn solution concentration the tissue concentration of *A. pseudoplatanus* is greater than that of *U. dioica*, but at higher concentrations the converse is true, increasing from a 3 fold to a 13 fold difference at the highest solution concentration. The relationships between solution and tissue concentration between the species were also different; *U. dioica* having a steep linear relationship whilst for *A. pseudoplatanus* the Zn tissue concentration reached a plateau at approximately 160 mg/kg. This suggests different responses to Zn between the two species. *U. dioica* continues to accumulate Zn until a toxic concentration is reached and the plant can no longer survive. In the present experiment this occurred to plants grown in the 41.9 mg Zn/l solution. In contrast, *A. pseudoplatanus* is able to limit transport of Zn into its above-ground tissue and therefore survive in media containing higher concentrations of Zn. However, the increasing difference between the water-soluble concentrations in *U. dioica* or phloem concentrations in *A. pseudoplatanus* and their bulk

tissue concentrations with increasing Zn exposure suggests that both species are able to limit the transport of Zn in their above-ground tissues.

Previous studies investigating the transfer of metals into aphids have used wheat grown in sewage sludge amended soils. In these studies the Zn concentrations in the plant tissue were substantially lower (<150 mg/kg; Green et al., 2003; Green et al., 2006; Merrington et al., 1997a. Merrington et al., 1997b; Winder et al., 1999) than those found in *U. dioica* in the current study and more comparable to those in *A. pseudoplatanus*. Despite this, the concentrations of Zn in *M. carnosum* reported in the current study are similar to those found in these previous studies, which used different aphid species (Green et al., 2003; Green et al., 2006; Merrington et al., 1997a. Merrington et al., 1997b; Winder et al., 1999). In contrast, the concentrations in *D. platanoidis* were generally two to three times greater, even at the lowest Zn solution concentration. All of these studies found that Zn accumulated in the aphids *Rhopalosiphum padi* and *Sitobian avenae* feeding on wheat. In our study, from the total plant concentrations it appeared that *M. carnosum* was not accumulating Zn as the *U. dioica* bulk tissue concentration from the 18.0 mg Zn/l solution was approximately 2100 mg/kg and the aphid concentration was 131 mg/kg. However, the analysis of the water-soluble tissue extract suggests that *M. carnosum* may actually be exposed to much lower concentrations. Zn concentrations in *D. platanoidis* were greater, at around 375 mg/kg, than both the total plant and the phloem concentrations of 160 and 6.2 mg/kg respectively.

It has been reported that Zn is concentrated in the stem tissue as well as the roots (Haslett et al., 2001) and is readily transported in the phloem of *A. pseudoplatanus* (Dollard and Lepp, 1980) and wheat (Haslett et al., 2001; Riesen and Feller, 2005). Aphids feed directly on the phloem sap (Dixon, 2005) and are therefore exposed to the Zn in this solution. The chemical form that Zn takes in the phloem is not well understood (Grusak et al., 2007), although it is likely to be in a soluble form, bound to chelators, amino acids and/or organic acids and it is also unclear whether the Zn is transported apoplastically or symplastically (Grusak et al., 2007). Studies on barley have shown that most of the Zn in the roots is in a soluble form. However, Zn in the leaves is mainly located in the mesophyll cells and, to a lesser extent the epidermal cells. The Zn in the apoplastic solution (97%) is mainly bound to cell walls (Grusak et al., 2007). The ability of *A. pseudoplatanus* to limit Zn in its above-

ground biomass, and because the transfer of Zn to the phloem is regulated by the requirements of plants (Haslett et al., 2001), may explain why, in this species, the concentrations in the phloem are similar regardless of the exposure to the plant or plant tissue concentrations. Water-soluble concentrations of Zn in *U. dioica* are much lower than the total plant concentrations, suggesting that the Zn within this species is also bound in the plant tissue. However, in *U. dioica*, the water-soluble Zn concentration did increase with Zn exposure which may suggest that this species is not as adept at limiting Zn transport or that it has a greater Zn requirement.

Although it appears that both aphid species bioaccumulated Zn, the concentrations in *M. carnosum* were smaller than those for *D. platanoidis* despite an increased level of exposure. This may be because the duration of exposure of *D. platanoidis* was double that for *M. carnosum*. Alternatively, *M. carnosum* may be able to regulate Zn; Crawford et al. (1995) found that *Aphis fabae* on broad beans (*Vicia faba*) were able to regulate Cu by excretion in honeydew. Unfortunately, it proved impossible to obtain sufficient quantities of honey dew for analysis in the present study. The Zn concentrations in *M. carnosum* were comparable with those found in other studies, whereas those in *D. platanoidis* were greater. This, coupled with the fact that the concentrations in *D. platanoidis* were greater even when *A. pseudoplatanus* was watered with the control solution suggest that this species may simply have greater Zn concentrations (or requirements) than have previously been reported in other aphid species.

The greater Zn concentration in *D. platanoidis* has important implications, both for the estimation of risk to higher organisms and the modelling of food-chain transfer as it highlights the, often substantial, differences between biological species. When the ladybird *Coccinella septempunctata*, lacewing *Chrysoperla carnea* and carabid beetle *Bembidion lampros* were fed aphids with Zn concentrations ranging between 163-249, 104-188 and 60-116 mg/kg respectively their corresponding tissue concentrations were between 184-217, 105-249 and 99-112 respectively (Green et al., 2003; Green et al., 2006; Winder et al., 1999). This suggests that, although only in the lacewing was Zn accumulated, the tissue concentrations of the predators of aphids are likely to reflect the tissue concentrations of their prey. Therefore species feeding on *D. platanoidis* may be exposed to higher concentrations of Zn in their diet

than those feeding on other species of aphid. This demonstrates the importance of species specificity in modelling food-chain transfer in terrestrial ecosystems.

## 5. Conclusions

This study demonstrates the importance of species-specify in ERA and food-chain transfer modelling. *A. pseudoplatanus* appears to be able to regulate the Zn concentration its above-ground biomass which results in tissue concentrations that are unaffected by Zn exposure at the concentrations tested. This results in a constant exposure to *D. platanoidis*. *U. dioica*, however, continues to accumulate Zn in its above-ground tissue at concentrations more than ten times greater than those measured in *A. pseudoplatanus*, resulting in an increasing level of exposure to *M. carnosum*. Despite the greater plant Zn concentrations in *U. dioica* compared with *A. pseudoplatanus*, *M. carnosum* had Zn concentrations that were approximately one third of those in *D. platanoidis*.

## Acknowledgements

The authors wish to thank Sylvia Cowdry for assisting with experimental work and Alberto Morales from the Environmental and Human Science Division Laboratory, Forest Research for sample analysis and James Morison. This work was funded by EPSRC as part of the Pollutants in the Urban Environment (PUrE) research consortium (Grant Number EP/C532651/2).

## 6. References

- Bryns, G., Crane, M. 2002. Assessing risks to ecosystems from land contamination. R&D Technical Report P299. Environment Agency, Bristol.
- USEPA. 2003. Multimedia, multipathway and multireceptor risk assessment (3MRA) modeling system. Volume II: Site-based, regional and national data. USEPA, Washington DC.
- USEPA. 2002. TRIM:FaTE Technical Support Document. Volume II: Description of chemical transport and transformation algorithms. USEPA, North Carolina.
- Edwards, S.C., MacLeod, C.L., Lester, J.N. 1998. The bioavailability of copper and mercury to the common nettle (*Urtica dioica*) and the earthworm *Eisenia fetida* from contaminated dredge spoil. Water, Air and Soil Pollution 102, 75-90.

- 350 Barta, M., Cagán, L. 2003. Entomophthoralean fungi associated with the common nettle aphid  
 351 (*Microlophium carnosum* Buckton) and the potential role of nettle patches as  
 352 reservoirs for the pathogens in landscape. *Journal of Pest Science* 76, 6-13.
- 353 Davis, B.N.K. 1991. *Insects on nettles*. Richmond Publishing, Slough, UK.
- 354 Sinnett, D., Hodson, M.E., Hutchings, T.R. (In press). Food-chain transfer of cadmium and  
 355 zinc from contaminated *Urtica dioica* to *Helix aspersa* and *Lumbricus terrestris*.  
 356 *Environmental Toxicology and Chemistry*.
- 357 Gilbert, O.L. 1991. *The Ecology of Urban Habitats*. Chapman and Hall, London.
- 358 Dixon, T. 2005. *Insect Herbivore-Host Dynamics: Tree dwelling aphids*. Cambridge University  
 359 Press, Cambridge.
- 360 Moffat, A.J., McNeill, J.D. 1994. Reclaiming disturbed land for forestry. Forestry Commission  
 361 Bulletin. 110. HMSO, Edinburgh.
- 362 Crawford, L.A., Hodkinson, I.D., Lepp, N.W. 1995. The effects of elevated host-plant cadmium  
 363 and copper on the performance of the aphid *Aphis fabae* (Homoptera: Aphididae).  
 364 *Journal of Applied Ecology* 32, 528-535.
- 365 Merrington, G., Winder, L., Green, I. 1997a. The uptake of cadmium and zinc by the bird-  
 366 cherry oat aphid *Rhopalosiphum padi* (Homoptera: Aphididae) feeding on wheat  
 367 grown on sewage sludge amended agricultural soil. *Environmental Pollution* 96, 111-  
 368 114.
- 369 Green, I., Merrington, G., Tibbet, M. 2003. Transfer of cadmium and zinc from sewage sludge  
 370 amended soil through a plant-aphid system to newly emerged adult ladybirds  
 371 (*Coccinella septempunctata*). *Agriculture, Ecosystems and Environment* 99, 171-178.
- 372 Braun, S., Flückiger, W. 1985. Increased population of the aphid *Aphis pomi* at a motorway:  
 373 Part 3 - The effect of exhaust gases. *Environmental Pollution* 39, 183-192.
- 374 Rotheray, G.E. 1989. *Aphid predators*. Richmond Publishing, Slough, UK.
- 375 Kean, J.M., Müller, C.B. 2004. Can competition explain local rarity of a nettle aphid?  
 376 *Ecological Entomology* 29, 706-710.
- 377 Hoagland, D.R., Arnon, D.I. 1941. *The water-culture method for growing plants without soil*.  
 378 *Miscellaneous Publications No. 3514*. California Agricultural Experiment Station,  
 379 Berkeley, CA, USA

- 380 Thornber, J.P., Northcote, D.H. 1961. Changes in the chemical composition of a cambial cell  
 381 during its differentiation into xylem and phloem tissue in trees. *Biochemical Journal*  
 382 81, 449-455.
- 383 Chapman, H.D., 1967. Plant analysis values suggestive nutrient status of selected crops, in:  
 384 Hardy, G.W. (Ed.), *Soil testing and plant analysis: Plant analysis part II*. Soil Science  
 385 Society of America, Wisconsin
- 386 Kilbride, C., Poole, J., Hutchings, T.R. 2006. A comparison of Cu, Pb, As, Cd, Zn, Fe, Ni and  
 387 Mn determined by acid extraction/ICP-OES and ex situ field portable X-ray  
 388 fluorescence analysis. *Environmental Pollution* 143, 16-23.
- 389 Genstat. 2005. *The Guide to GenStat Release 8.1 Part 2: Statistics*. VSN International,  
 390 Oxford, UK.
- 391 Hobbelen, P.H.F., Koolhaas, J.E., van Gestel, C.A.M. 2004. Risk assessment of heavy metal  
 392 pollution for detritivores in floodplain soils in the Biesbosch, the Netherlands, taking  
 393 bioavailability into account. *Environmental Pollution* 129, 409-419.
- 394 Tack, F.M., Verloo, M.G. 1996. Metal contents in stinging nettle (*Urtica dioica* L.) as affected  
 395 by soil characteristics. *Science of the Total Environment* 192, 31-39.
- 396 Jones, D.T. 1991. *Biological monitoring of metal pollution in terrestrial ecosystems*. PhD  
 397 Thesis. University of Reading, UK.
- 398 Mertens, J., Vervaeke, P., De Schrijver, A., Luysaert, S. 2004. Metal uptake by young trees  
 399 from dredged brackish sediment: limitations and possibilities for phytoextraction and  
 400 phytostabilisation. *Science of the Total Environment* 326, 209-215.
- 401 Kabata-Pendias, A., Pendias, H. 2001. Elements of Group II, in: Kabata-Pendias A, Pendias  
 402 H. (Eds.), *Trace Elements in Soils and Plants*, 3rd Edition. CRC Press Ltd., Boca  
 403 Raton, Florida, pp 123-164.
- 404 Merrington, G., Winder, L., Green, I. 1997b. The bioavailability of Cd and Zn from soils  
 405 amended with sewage sludge to winter wheat and subsequently to the grain aphid  
 406 *Sitobion avenae*. *Science of the Total Environment* 205, 245-254.
- 407 Winder, L., Merrington, G., Green, I. 1999. The tri-trophic transfer of Zn from the agricultural  
 408 use of sewage sludge. *Science of the Total Environment* 229, 73-81.

- 409 Green, I.D., Jeffries, C., Diaz, A., Tibbett, M. 2006. Contrasting behaviour of cadmium and  
410 zinc in a soil-plant-arthropod system. *Chemosphere* 64, 1115-1121.
- 411 Haslett, B.S., Reid, R.J., Rengel, Z. 2001. Zinc mobility in wheat: Uptake and distribution of  
412 zinc applied to leaves or roots. *Annals of Botany* 87, 379-386.
- 413 Dollard, G.J., Lepp, N.W. 1980. Differential mobility of lead and zinc in phloem tissue of  
414 sycamore (*Acer pseudoplatanus* L.). *Zeitschrift fur Pflanzenphysiologie* 97, 409-415.
- 415 Riesen, O., Feller, U. 2005. Redistribution of nickel, cobalt, manganese, zinc and cadmium  
416 via the phloem in young and maturing wheat. *Journal of Plant Nutrition* 28, 421-430.
- 417 Grusak, M.A., Pearson, J.N., Marentes, E. 2007. The physiology of micronutrient homeostasis  
418 in field crops. *Field Crop Research* 60, 41-56.

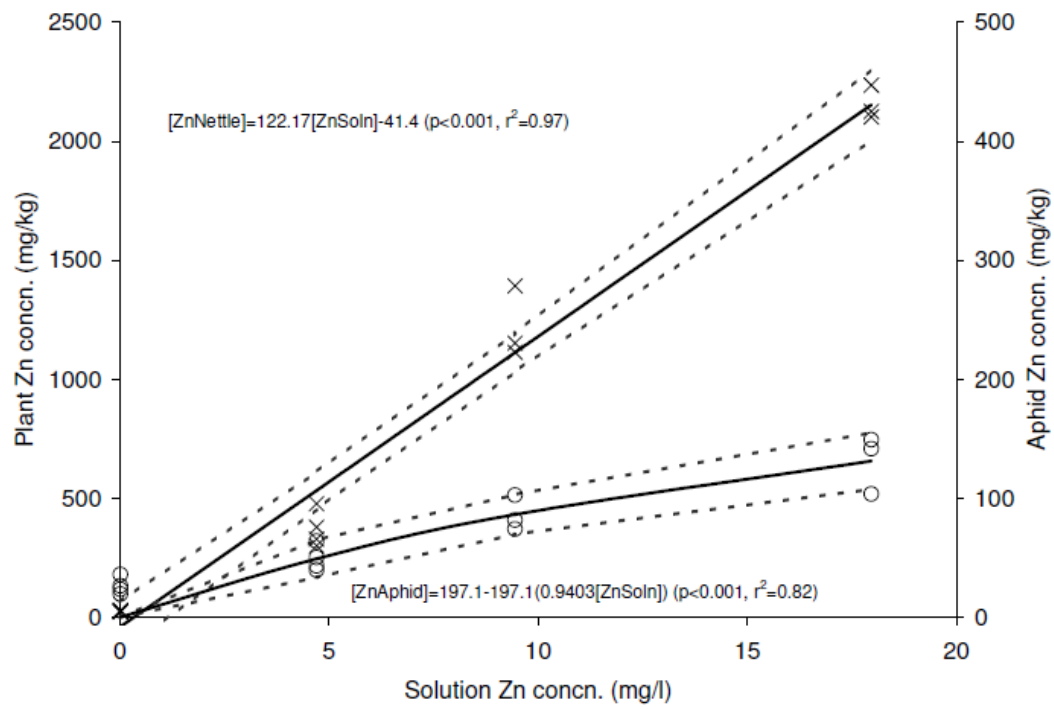


419 **Fig. 1.** Zn uptake models (solid lines), with 95% confidence intervals (dashed lines), and data  
 420 for the above-ground tissue of *Urtica dioica* (x; n=15; 54 d exposure) and the whole body of  
 421 *Microlophium carnosum* (o; n=15; 28 day exposure) after exposure to Zn. Where [ZnNettle],  
 422 [ZnAphid], [ZnSoln] is the concentration of Zn in the tissue of *U. dioica* (mg/kg dry weight), *M.*  
 423 *carnosum* (mg/kg dry weight) and the watering solution (mg Zn/l) respectively.

424 **Fig. 2.** Water-soluble Zn concentration in above-ground tissue of *Urtica dioica* (o; n=15;  
 425 mg/kg wet weight; 98 day exposure) and phloem tissue of *Acer pseudoplatanus* (x; n=15;  
 426 mg/kg wet weight; 98 day exposure) compared with the Zn concentrations of Hoagland's  
 427 solution in which the plants were grown.

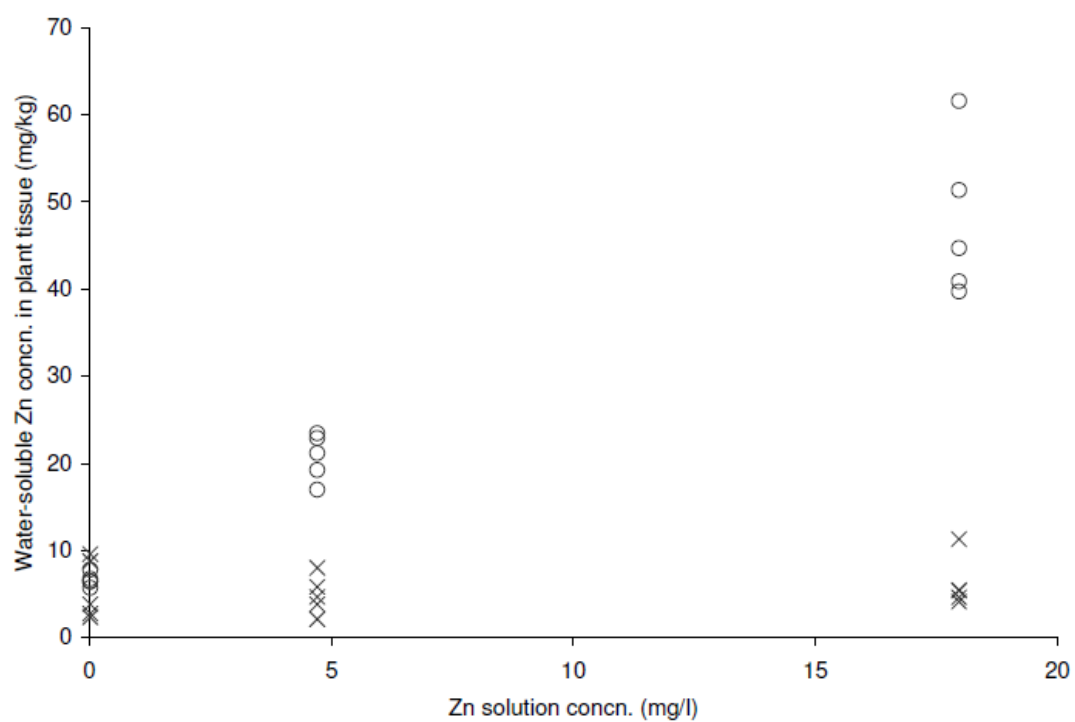
428 **Fig. 3.** Zn concentration in leaf tissue of *Acer pseudoplatanus* (x; n=25; mg/kg; 98 day  
 429 exposure) and in the whole body tissue of *Drepanosiphum platanoidis* (o; n=23; mg/kg; 14  
 430 day exposure) compared with the Zn concentrations of Hoagland's solution in which the *A.*  
 431 *pseudoplatanus* were grown. Zn uptake model (solid lines) for concentration in the leaves of  
 432 *A. pseudoplatanus*, with 95% confidence intervals (dashed lines) where [ZnSyc] and [ZnSoln]  
 433 is the concentration of Zn in the leaf tissue of *A. pseudoplatanus* (mg/kg dry weight) and the  
 434 watering solution (mg Zn/l) respectively.

435 Figure 1



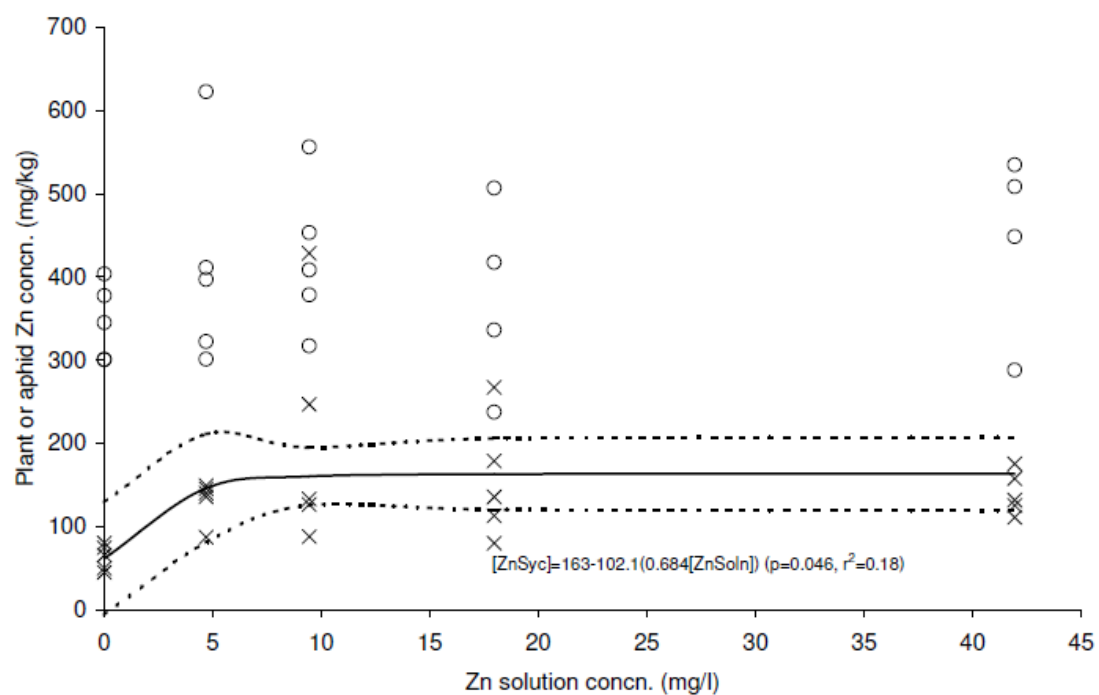
436

437 Figure 2



438

439 Figure 3



440